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ERYSIPHE SYMPHORICARPI (ERYSIPHALES), THE FIRST RECORD IN UKRAINE

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Abstract. A powdery mildew on an ornamental plant *Symphoricarpos albus* (Caprifoliaceae) was found in Kyiv (Ukraine) in October 2015. The causal agent was mainly in the anamorphic state, but the teleomorph in the form of several fruiting bodies (chasmothecia) were present. Only one of these was almost mature. Its morphology suggested it belonged to the common North American species *Erysiphe* (*Microsphaera*) *symphoricarpi*. Two separate phylogenetic analyses using ITS and 28S rDNA sequences showed that the fungus on *S. albus* collected in Ukraine grouped with *E. symphoricarpi* collected in Hungary, UK, and USA with strong bootstrap supports (MP = 100%, ML = 100%). This is the first record of this species in Ukraine. It is assumed that the disease will not have a significant impact on green spaces, because the pathogen develops in late autumn and fruiting bodies are rare.

Key words: *Symphoricarpos*, powdery mildew fungi, molecular phylogeny, introduced species, North American fungus, ornamental planting, snowberry

Introduction

Over recent decades, the number of powdery mildew species in Ukraine has increased due to the introduction of alien powdery mildews of North American and East Asian origin. For example, species such as *Erysiphe palczewskii* (Jacz.) U. Braun & S. Takam. (Heluta, 1981; Heluta, Gorlenko, 1984), *E. vanbruntiana* (W.R. Gerard) U. Braun & S. Takam. (Heluta, 1981; Heluta, Gorlenko, 1981), *E. syringae-japonicae* (U. Braun) U. Braun & S. Takam. (Seko et al., 2008, 2011), *E. arcuata* U. Braun, V.P. Heluta & S. Takam. (Braun et al., 2006), *E. kenjiana* (Homma) U. Braun & S. Takam. (Heluta et al., 2009), *E. macleayae* R.Y. Zheng & G.Q. Chen (Heluta, Kravchuk, 2015; Heluta et al., 2016), and *Neoerysiphe geranii* (Y. Nomura) U. Braun (Heluta, 2001; Heluta et al., 2010) migrated from East Asia. On the other hand, some native North American species, *Erysiphe azaleae* (U. Braun) U. Braun & S. Takam., *E. elevata* (Burrill) U. Braun & S. Takam., *E. flexuosa* (Peck) U. Braun & S. Takam., *E. platani* (Howe) U. Braun & S. Takam., *Golovinomyces greeneanus*

(U. Braun) V.P. Heluta, and *Podosphaera amelanchieris* Maurizio, extended eastward and migrated to Ukraine via Western Europe (Heluta, Voytyuk, 2004; Heluta et al., 2004, 2009, 2013; Heluta, Korytnianska, 2011; Heluta, Hirylovich, 2016). Therefore, their first reports in Ukraine followed those from Western Europe.

In the 90s of the last century, *E. symphoricarpi* (Howe) U. Braun & S. Takam. parasitizing species of the genus *Symphoricarpos* Duhamel (Caprifoliaceae) was found in Western Europe (Kiss et al., 2002; Kiss, 2005). Until 2015, this fungus did not occur in Ukraine, despite the fact that various snowberry species are cultivated in the botanical gardens within the country and *S. albus* (L.) S.F. Blake is a common ornamental plant. In early October 2015, *S. albus* affected by powdery mildew was observed in green spaces adjacent to buildings in Pivdenna (South) Borshchahivka (Kyiv, Ukraine) (Fig. 1, a). The upper surface of leaves was covered with a thin layer of greyish mycelium with very scarce conidiophores. The lower surfaces of leaves were also covered with mycelium, although very subtle and visible only under the dissection microscope.

The objective of this study was to identify the fungal species based on molecular and morphological features.

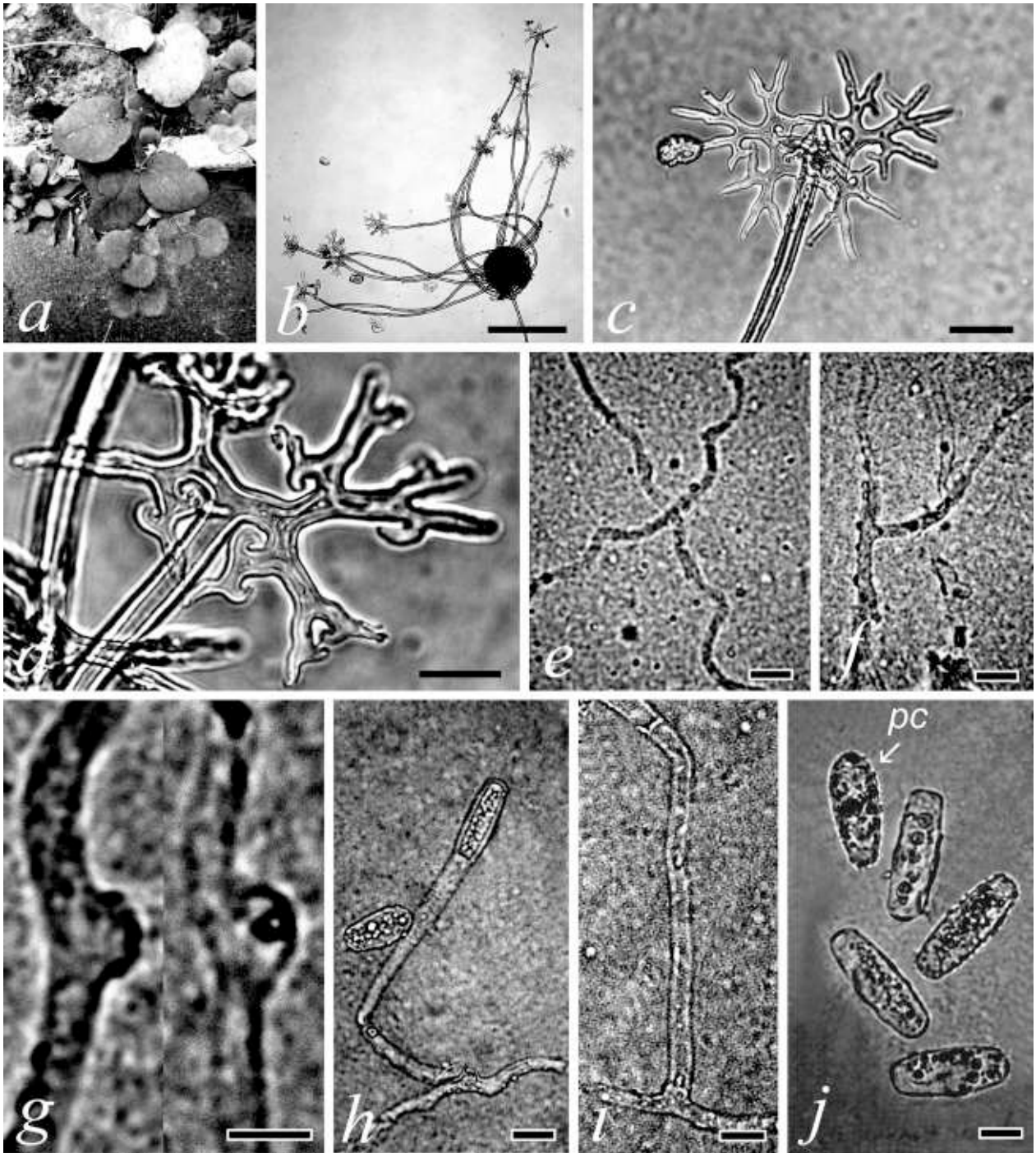


Fig. 1. *Erysiphe symphoricarpi* on *Symphoricarpos albus*: *a* – leaves of the host plant affected by the fungus; *b* – chasmothecium; *c*, *d* – apical part of the appendage with straight (*c*) and recurved (*d*) tips; *e*, *f* – hyphae (*e* – twisted, *f* – branched at an angle of 90°); *g* – hyphal appressoria; *h* – conidiophore; *i* – foot cell of the conidiophore; *j* – primary (*pc*) and secondary conidia. Bars: *b* – 200 μm , *c* – 20 μm , *d*–*f*, *h*–*j* – 10 μm , *g* – 5 μm

Materials and methods

Molecular phylogenetic analysis

The nucleotide sequences of the 5'-end of the nuc 28S *rDNA* (including domains D1 and D2) and the nuc *rDNA* ITS1-5.8S-ITS2 (ITS) were determined by the procedure described by S. Takamatsu et al. (2013). The sequence determined in this study was deposited in DNA Data Base of Japan (DDBJ) under the accession number LC167137. Newly determined sequence was aligned with other sequences of the genus *Erysiphe* retrieved from DNA databases using MUSCLE (Edgar, 2004) implemented in MEGA 6 (Tamura et al., 2013). Alignments were further manually refined using the MEGA6 program and deposited in TreeBASE (<http://www.treebase.org/>) under the accession number S19509. Phylogenetic trees were obtained from the data with maximum parsimony (MP) and maximum likelihood (ML) analyses. MP-analyses were conducted by PAUP 4.0a146 (Swofford, 2002) with the heuristic search option using 'tree bisection-reconstruction' (TBR) algorithm with 100 random sequence additions to find the global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were also calculated. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analysis (Felsenstein, 1985) using 1000 replications with the step-wise addition option set as simple. The ML-analysis was done with raxmlGUI (Silvestro, Michalak, 2012) under a GTRGAMMA model. The BS supports and trees were obtained by running rapid bootstrap analysis of 1000 pseudo replicates followed by a query for the tree with the highest likelihood. BS supports of 70% or higher are shown.

Morphological study

The materials collected were dried between papers at 22–24 °C. Two herbarium specimens are deposited at the National Herbarium of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (KW 60921F and 70436F). The fungus was studied and photographed under a light microscope «Primo Star» (Carl Zeiss, Germany) with the camera «Canon A 300» and the software «AxioVision 4.7». The mycelium, conidiophores and conidia were removed from the surface of infected leaves by a transparent adhesive tape. To restore shape and size, a piece of tape

with these fungus structures was put in a droplet of 40% lactic acid solution on a microscope slide (sticky side up), covered with a cover glass, gently heated to boiling point, then examined under the light microscope. Only one chasmothecium was prepared and studied in a drop of distilled water.

Results and discussion

Molecular phylogenetic analysis

Two separate phylogenetic analyses were done in this study. For the first analysis, we used combined data set of ITS and 28S *rDNA* sequences to investigate phylogenetic placement of *E. symphoricarpi* in the genus *Erysiphe* (*Microsphaera* lineage). For the second analysis, we used ITS sequences to compare with the sequences of *E. symphoricarpi* collected in four countries. In the first analysis, an ITS + 28S *rDNA* combined data set consisted of 37 sequences and 1390 characters, of which 21 characters at the end of the ITS2 region were deleted from the analysis due to an ambiguous alignment. Of the remaining 1369 characters, 294 (21.5%) characters were variable and 161 (11.8%) characters were informative for parsimony analysis. A total of nine equally parsimonious trees with 682 steps were constructed by the MP-analysis. Tree topologies were almost consistent among the trees, except for branching orders of the terminal groups and branch lengths. One of the trees with the highest likelihood value is shown in Fig. 2. ML-analysis generated a tree topology almost identical to the MP tree, and only BS support values are shown on the MP tree. The fungus (KW 60921F) on *Symphoricarpos albus* collected in Ukraine grouped with *E. symphoricarpi* (LC009970) collected in UK with strong bootstrap supports (MP = 100%, ML = 100%). *Erysiphe lonicerae* (LC010020) ex *Lonicera* sp. was sister to this group (MP = 98%, ML = 98%).

The second analysis was conducted using three ITS sequences from *E. symphoricarpi* collected from USA, UK, and Hungary. Two sequences from *E. lonicerae* were used as outgroup. The data set consisted of six sequences and 554 characters, of which 12 (2.2%) characters were variable and 10 (1.8%) characters were informative for parsimony analysis. A single most parsimonious tree was constructed by the MP-analysis (Fig. 3). ML-analysis generated a tree topology identical to the MP tree. The fungus (KW 60921F) on *S. albus* collected in Ukraine grouped with three *E. symphoricarpi* sequences with strong bootstrap supports (MP = 100%, ML = 100%).

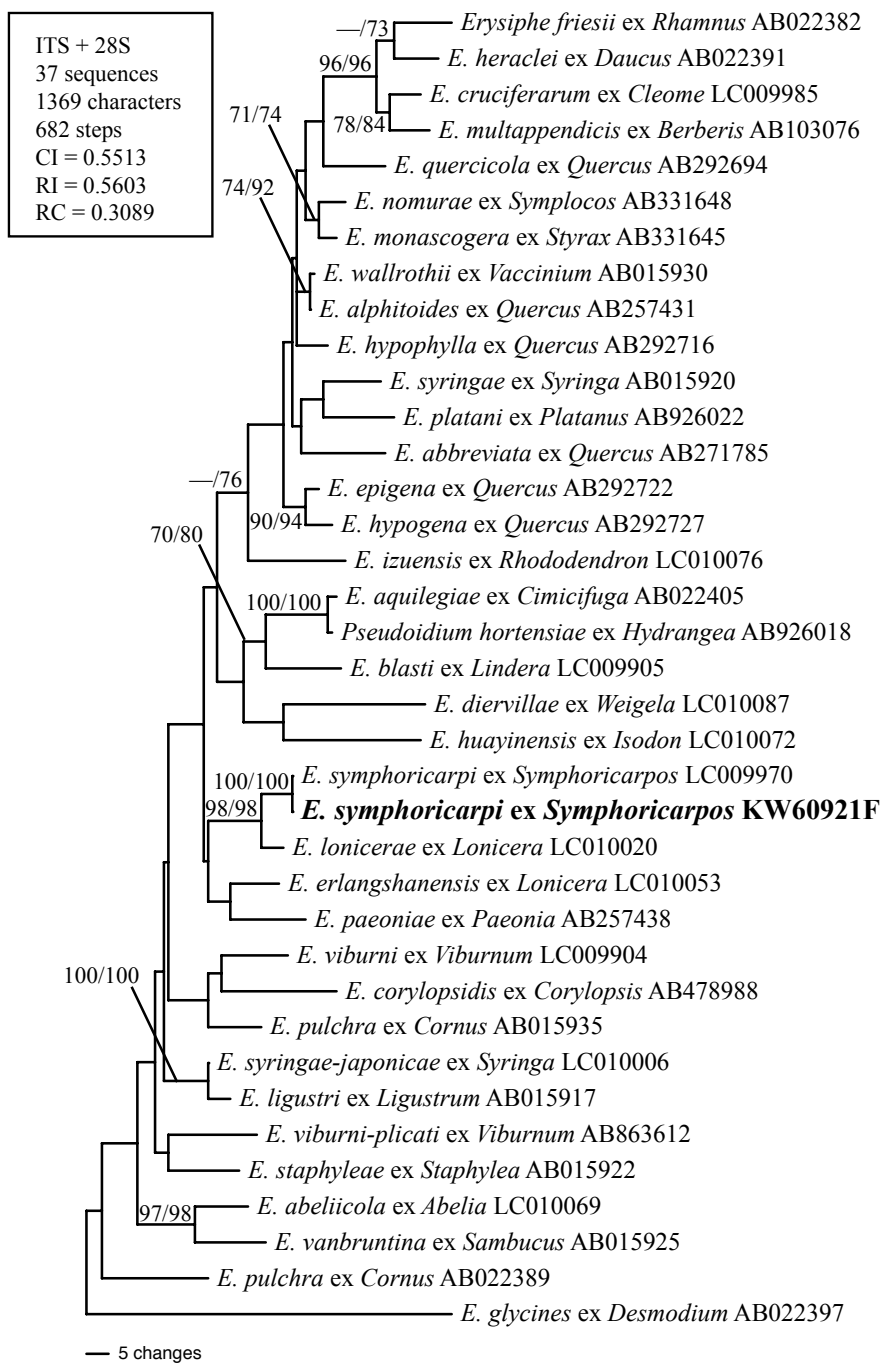


Fig. 2. Phylogenetic analysis of combined data of the divergent domains D1 and D2 sequences of the 28S *r*RNA gene and ITS region for 37 sequences from the genus *Erysiphe*. This tree is a phylogram of one of the nine equally parsimonious trees with 682 steps, which were found using a heuristic search. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch of the tree. BS ($\geq 70\%$) values produced by the maximum parsimony (MP) and maximum likelihood (ML) methods are shown on/under the respective branch. The sequence determined in this study is shown in bold phase

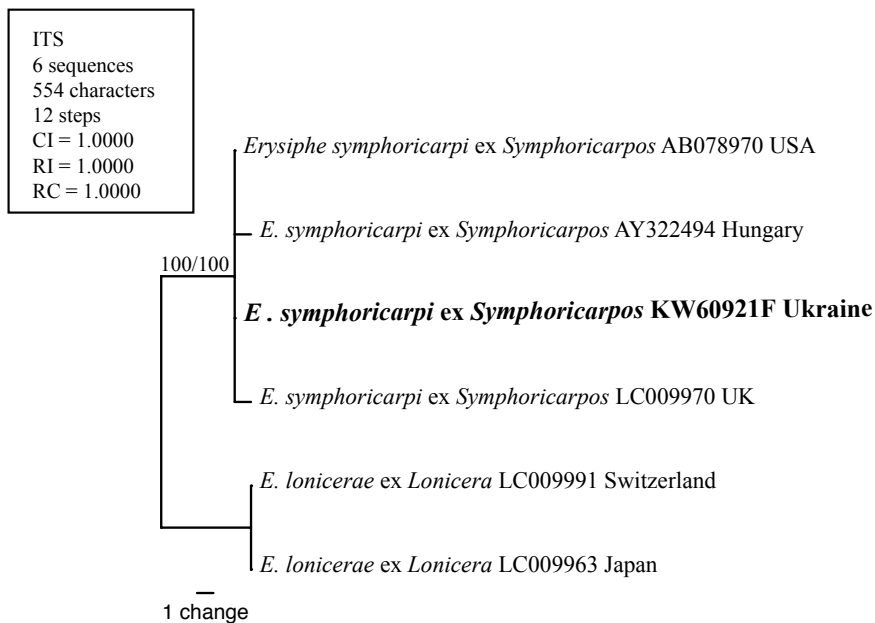


Fig. 3. Phylogenetic analysis of rDNA ITS region for six sequences from *Erysiphe symphoricarpi* and *E. lonicerae*. This tree is a single most parsimonious tree with 12 steps, which were found using a heuristic search. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch of the tree. BS ($\geq 70\%$) values produced by the maximum parsimony (MP) and maximum likelihood (ML) methods are shown on the respective branch. The sequence determined in this study is shown in bold phase

Morphological study

As a result of careful examination of our samples, we have seen only a few primordia and a single fruit body with mature appendages. The chasmothecium had long appendages with dichotomously divided rather loose apices (Fig. 1, *b–d*). Thus, molecular peculiarities, morphological characteristics of this fungus, and its host plant coincided with those of *E. symphoricarpi* given in the literature (Kiss et al., 2002; Braun, Cook, 2012). Morphological characteristics of the fungus are provided below.

***Erysiphe symphoricarpi* (Howe) U. Braun & S. Takam., Schlechtendalia 4: 14. 2000 (Fig. 1)**

Syn.: *Microsphaera symphoricarpi* Howe, in Bessey, Bull. Torrey bot. Club 5: 3. 1874. – *Trichocladia symphoricarpi* (Howe) Jacz., Karm. Opred. Grib., Vyp. 2. Muchn.-rosj. griby (Leningrad): 291. 1927

Mycelium in white or greyish patches of varying size merging into a continuous coating. Mycelia consisted of ectophytic hyphae and scarce 3–4 celled conidiophores that produced single conidia. Superficial hyphae often twisted, 3–6 μm wide, branched generally at an angle of 90° . Hyphal appressoria entire, 6–9 μm in diam. Conidiophores very scarce, consisting of a mainly cylindrical and very long (up to 105 μm) foot cell, usually followed by two other cells, 100–194 μm long. Conidia formed singly, mainly cylindrical, occasionally slightly ellipsoid, 24–36 \times 8.5–13.5 μm ,

with a length/breadth ratio of 2.0–3.1, germinated with a single terminal germ tube. Chasmothecium 104 μm in diam., with 16 appendages 2–6 times as long as the width of chasmothecium (up to 655 μm long). Appendages with dichotomously branched apices, the tips slightly recurved or straight. Chasmothecium contained only immature asci.

Specimens studied: Ukraine, Kyiv, Pivdenna Borshchahivka, 9 Bulgakov Str., on *Symphoricarpos albus*, 02.10.2015, V.P. Heluta (*KW* 60921F) and 10.10.2015, V. P. Heluta (*KW* 70436F).

Our results indicate that the appendages of the studied fungus have some minor differences compared to those in previously known descriptions (Salmon, 1900; Braun, Cook, 2012). As illustrated above (Fig. 1, *d*), some tips of the terminal branches have a tendency to be recurved. According to U. Braun and R.T.A. Cook (2012), *E. lonicerae* DC. may also occasionally have appendages with curved tips. Since this species is genetically close to *E. symphoricarpi* (see Molecular phylogenetic analysis), such a difference within our pathogen is acceptable. In addition, foot cells of conidiophores of the Ukrainian specimens are much longer than those in the description provided by U. Braun and R.T.A. Cook (2012). Other features of our specimens fall within the range of variation of *E. symphoricarpi* presented in the literature (Kiss et al., 2002; Braun, Cook, 2012).

Discussion

Nine species of the genus *Symphoricarpos* of American origin are cultivated in Ukraine, but only *S. albus* is a well-known ornamental plant (Kokhno et al., 1986). Some snowberry species affected by powdery mildews, such as *E. diffusa* (Cooke & Peck) U. Braun & S. Takam., *E. penicillata* (Wallr.) Link, *E. symphoricarpi*, or *Podosphaera clandestina* (Wallr.) Lév., have been reported in the USA (Farr et al., 1989). Nevertheless, U. Braun and R.T.A. Cook (2012) listed only *E. symphoricarpi* on *Symphoricarpos*. Concerning the American *Erysiphe* species on *Symphoricarpos* spp., *E. diffusa* is morphologically very similar to *E. symphoricarpi*, but is confined to hosts of *Fabaceae*, and *E. penicillata* is confined to *Alnus*. *Podosphaera clandestina* is known to parasitize rosaceous hosts; so, records of this species on *S. albus* are also very doubtful. Consequently, only one powdery mildew species, *E. symphoricarpi*, apparently occurs on snowberry plants.

As mentioned above, *Erysiphe symphoricarpi* was introduced to Europe in the 90s of the last century. Its distribution in this part of the world was investigated in detail by L. Kiss et al. (2002). The fungus was first registered in Germany as *Microsphaera loniceriae* (DC.) G. Winter (Foitzik, 1990; Jage et al., 2010). Subsequently, in 1990, 1996, 1997 and 1999–2001, *E. symphoricarpi* was found in England and Scotland (Kiss et al., 2002; Henricot, 2009), in 1996 in Switzerland and in 2002 in Germany (Kiss et al., 2002). Almost the same time, it was also reported from Poland (Czerniawska, Madej, 1998; Czerniawska et al., 2000) and soon after recorded in Hungary (Kiss et al., 2002; Szentivanyi et al., 2004). It is noteworthy that only one sample collected in Germany contained fruiting bodies, all the rest represented the anamorph.

Despite the fact that *E. symphoricarpi* migrated to Europe a long time ago, the fungus was found in Ukraine only last year. Therefore, in contrast to other species introduced from America, especially such as *E. necator* Schwein. or the recently introduced *E. flexuosa* and *Podosphaera amelanchieris*, this one may have spread further eastward only slowly and we agree with Kiss (2005) that *E. symphoricarpi* is not a dangerous invasive powdery mildew fungus.

Thus, a newly introduced species of North American origin, *E. symphoricarpi*, was found to occur on an ornamental plant, *Symphoricarpos albus*, in Ukraine. Since the fungus develops in late autumn and fruiting bodies are rare, we suggest that this pathogen will not have a significant negative impact on parks.

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Повідомляється, що в 2015 р. в Україні була зареєстрована борошніста роса на декоративній рослині *Symphoricarpos albus (Caprifoliaceae)*. Гриб-збудник хвороби формував переважно конідіальну стадію, однак було знайдено і декілька плодових тіл, лише одне з них було майже зрілим. Його морфологічні ознаки свідчать, що знайдений гриб належить до північно-американського *Erysiphe (Microsphaera) symphoricarpi*. Був також здійснений філогенетичний аналіз із залученням ITS та 28S рДНК-последовностей. У результаті показано, що зразок з України з великою достовірністю об'єднується в одну групу з трьома зразками *E. symphoricarpi*, зібраними у Великій Британії, Угорщині та США. Це перша знахідка даного гриба в Україні. Він виявлений в Києві, лише в одному локалітеті. Допускається, що гриб не буде завдавати значної шкоди зеленим насадженням України, оскільки він розвивається пізно восени і майже не утворює плодових тіл.

Ключові слова: *Symphoricarpos*, борошністоросяні гриби, молекулярна філогенія, інтродукований вид, північно-американський гриб, декоративне насадження, сніжноягідник

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Сообщается, что в 2015 г. в Украине была зарегистрирована мучнистая роса на декоративном растении *Symphoricarpos albus (Caprifoliaceae)*. Гриб-возбудитель заболевания развивался преимущественно на анаморфной стадии, однако было найдено и несколько плодовых тел. Только одно из них было почти зрелым. Его морфологические признаки свидетельствуют о том, что найденный гриб принадлежит к северно-американскому *Erysiphe (Microsphaera) symphoricarpi*. Был также проведен филогенетический анализ с использованием ITS и 28S рДНК-последовательностей. В результате показано, что образец из Украины с большой достоверностью объединяется в одну группу с тремя образцами *E. symphoricarpi*, собранными в Великобритании, Венгрии и США. Это первая находка данного гриба в Украине. Он обнаружен в Киеве, только в одном локалитете. Предполагается, что гриб не будет приносить значительного вреда зеленым насаждениям Украины, поскольку развивается поздней осенью и практически не образует плодовых тел.

Ключевые слова: *Symphoricarpos*, мучнисторосяные грибы, молекулярная филогенія, интродуцированный вид, северно-американский гриб, декоративное насаждение, снежноягодник